Biological membranes, cell organelles, whole cells and viruses can be fractionated by CCD in the same kind of systems. In this case, however, the particles partition between the two liquid phases and the interface between them. The CCD is therefore usually carried out using a stationary interface. This is achieved by using a smaller volume of the lower phase than is needed to fill the lower cavities. Therefore, a portion of the upper phases will also be stationary. The G value satisfying eqn [5] is in this case defined as the amount of a pure compound, at equilibrium, in the mobile part of the upper phase divided by the amount of the compound in the rest of the system (stationary upper phase, interface and lower phase). Examples of CCD of proteins and of chloroplasts, the photosynthetic organelle in green plant cells, are given in **Figure 7**.

See also: **II/Chromatography:** Countercurrent Chromatography and High-Speed Countercurrent Chromatography: Instrumentation.

Further Reading

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Solid-Phase Extraction

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Solid-phase extraction is a method used to isolate analytes from a gas, fluid or liquid by their transfer to and retention on a solid-phase sorbent. After separation of the sorbent from the sample the analytes are recovered by elution using a liquid or fluid, or by thermal desorption into the gas phase. If the analytes are recovered from the sorbent in a final volume that is only a fraction of the sample volume, then concentration as well as isolation is achieved. In addition, if the sorption step, any subsequent rinse steps, and the elution conditions are selective for retention and recovery of the analyte, then matrix simplification is achieved. Isolation, concentration and matrix simplification are the primary goals of solid-phase extraction.

Probably the earliest application of solid-phase extraction was the use of charcoal-filled columns in the 1950s to isolate organic contaminants from surface waters for toxicity evaluation. The large volume of water generally sampled (more than 1000 L over several days) precluded the use of liquid–liquid extraction techniques. The subsequent evolution of solid-phase extraction techniques is summarized in **Figure 1**.

The introduction of macroreticular porous polymers in the early 1970s was responsible for rekindling interest in solid-phase extraction and extending its scope to air sampling and the isolation of drugs from biological fluids. These sorbents had reasonable mechanical strength compared with gels, a large surface area and sample capacity, low water retention, and gave high sample recoveries by solvent desorption. Compared with carbon the overall analyte recovery was generally better and irreversible adsorption and catalytic activity greatly diminished. These

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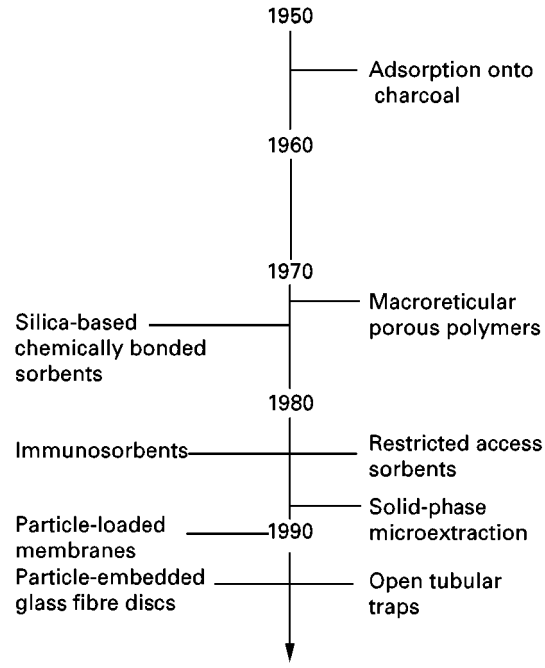

Figure 1 Time line showing the general evolution of solid-phase extraction techniques.

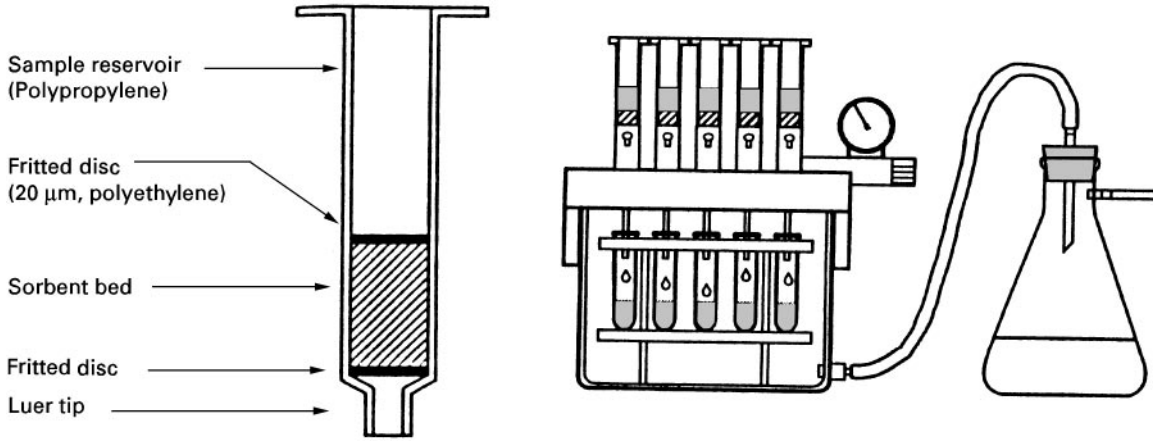

Figure 2 Schematic diagram showing the typical construction of a solid-phase extraction cartridge and a vacuum manifold for parallel sample processing.

properties, together with a reduction in the amount of material needed for identification due to improved instrumentation, resulted in the general use of small columns, similar in size to those in use today. Porous polymers with high thermal stability and low water retention revolutionized the room temperature sorbent extraction of volatile organic compounds from air or purge gas from water samples. Trapped compounds were thermally desorbed directly into a gas chromatograph for analysis. Automated systems based on the above process are used for routine analysis today.

Solid-phase extraction for liquid samples became a widely used laboratory technique with the introduction of disposable sorbent cartridges containing porous, siloxane-bonded silica particles, sized to allow sample processing by gentle suction (**Figure 2**). A typical solid-phase extraction cartridge consists of a short column (generally an open syringe barrel) containing a sorbent with a nominal particle size of 50–60 μm, packed between porous metal or plastic frits. A large number of sorbents are in use today corresponding to the desire for general purpose, class-specific and even compound-specific extractions.

Slow sample processing rates for large sample volumes, low tolerance to blockage by particles and sorbed matrix components, and problems arising from the low and variable packing density of cartridge devices spawned the development of alternative sampling formats based on disc technology. At least three different designs for solid-phase extraction discs are offered commercially today. The particle-loaded membranes consist of a web of polytetrafluoroethylene (PTFE) microfibrils, suspended in which are sorbent particles of about 8–10 μm diameter. The membranes are flexible with a homogeneous structure containing 80% (w/w) or more of sorbent particles formed into circular discs 0.5 mm thick with diameters from 4 to 96 mm. For general use they are supported on a sintered glass disc (or other support) in a standard filtration apparatus using suction to generate the desired flow through the membrane (**Figure 3**). Particle-embedded glass fibre discs contain 10–30-μm sorbent particles woven into a glass fibre matrix. The small diameter discs are rigid and self-supporting, while the larger diameter discs require a supporting structure. Speediscs® (**Figure 4**) consist of a sandwich of 10-μm sorbent particles held between two glass-fibre filters, with a screen to hold the filters in place. Disc technology has contributed directly to the automation of solid-phase extraction through the development of the multiwell extraction plate (**Figure 5**), which is used for the clean-up of samples in high-throughput screening techniques for drug development. Direct coupling of solid-phase extraction and high pressure liquid chromatography for on-line sample processing and analysis is now routine and the direct coupling of solid-phase extraction and gas chromatography for the analysis of liquid samples has moved beyond the research phase. Several research groups have demonstrated the direct coupling of solid-phase extraction and electrophoretic and thin-layer chromatographic separation techniques.

Replacement for Liquid–Liquid Extraction

Solid-phase extraction was introduced as a replacement for liquid–liquid extraction to give a practical and economic solution to the real and perceived problems associated with solvent extraction techniques. Liquid–liquid extractions are labour

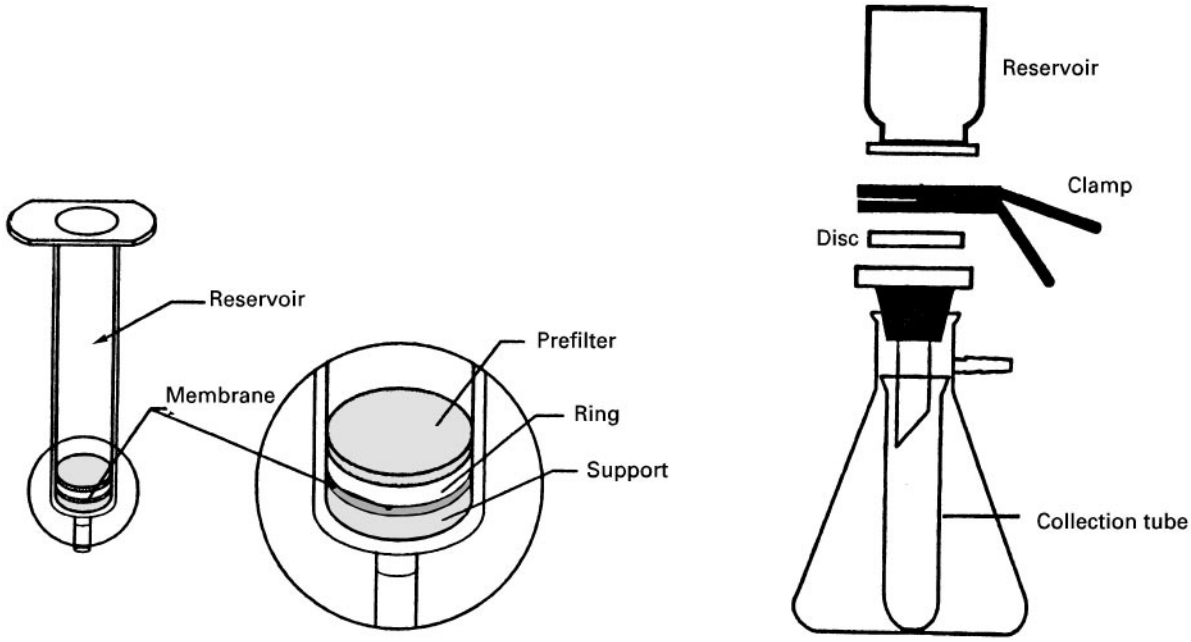

Figure 3 Typical cartridge and vacuum filtration formats for solid-phase extraction using discs.

intensive, difficult to automate, and frequently plagued by practical problems, such as emulsion formation. Liquid–liquid extractions also tend to consume large volumes of high purity solvents, which may have significant health hazards and disposal costs associated with their use. In contrast, solid-phase extraction benefits from lower intrinsic costs, reduced processing times, low solvent consumption and simpler processing procedures. Solid-phase extraction procedures are easily automated using robotics, or special purpose flow processing units that simultaneously extract samples and prepare them for automatic injection, or by using centrifugal analysers, which can batchwise process multiple samples. Solid-phase extraction is convenient for field sampling since it minimizes the transport and storage problems of bulk samples, which have to be returned to the laboratory for processing.

Solid-phase extraction techniques have their own, although different, problems to those of liquid–liquid

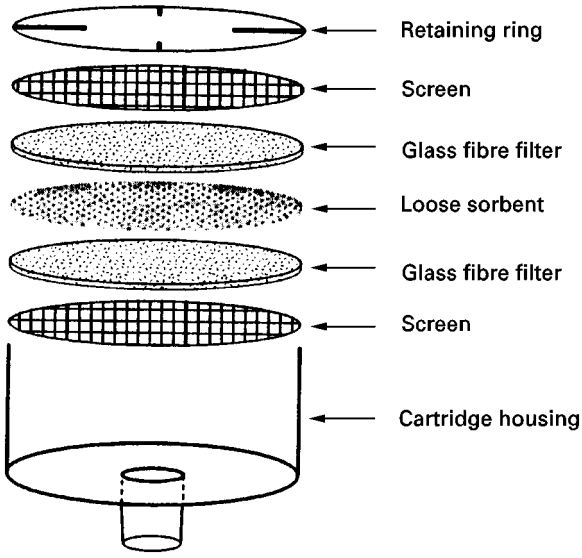

Figure 4 Exploded-view of the Speedisc® used for solid-phase, extraction.

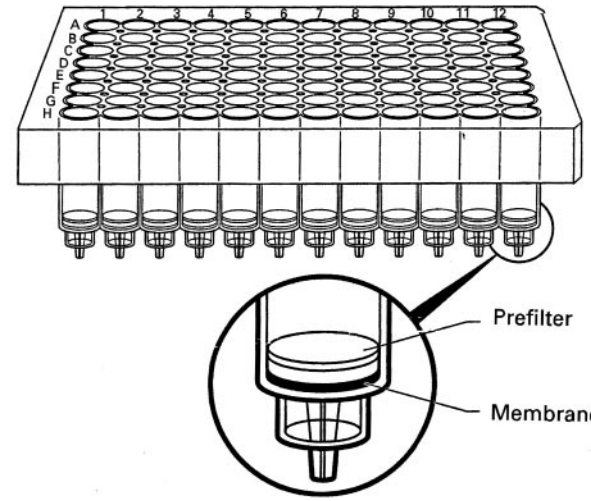

Figure 5 Multiwell plate for automated solid-phase extraction. (Reproduced with permission from Plumb RS, Gray RDM, and Jones CM (1997). Use of reduced sorbent bed and disc membrane solid-phase extraction for the analysis of pharmaceutical compounds in biological fluids, with applications in the 96-well format. *Journal of Chromatography B* 694:123–133.)

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extraction. The sorption properties of manufactured sorbents are not as reproducible as solvent properties. Basic drugs, for example, are often retained on silica-based, chemically bonded sorbents by a mixed retention mechanism involving non-specific sorption by the bonded phase and ion exchange interactions with accessible, dissociated silanol groups. The mixed retention mechanism can interfere in the recovery of analytes since solvent elution may be ineffective for removing ionically bound analytes, and the extent of binding through ionic sites can vary for different sorbent lots. Sorbents tend to have a higher level of contamination by manufacturing and packaging materials than do solvents. The chemical background from impurities may interfere in the subsequent analysis of the sample. Solvent rinsing of cartridges and discs and the running of blanks to establish background contamination levels diminishes sample throughput and adds significantly to solvent consumption and processing costs. Sample processing problems, such as column overloading, displacement and blocking of sorbent pores, easily go unnoticed, resulting in changes in analyte recovery. Sample overload and displacement are more important for extraction based on adsorption than for extraction based on absorption.

When choosing between liquid–liquid or solid-phase extraction for a particular problem, economic, as well as technical features, should be taken into consideration. In this sense, liquid–liquid and solid-phase extraction techniques should be considered complementary approaches, and although the general trend is towards the replacement of liquid–liquid extraction methods by solid-phase extraction, this is never likely to be a complete replacement.

Disc Versus Cartridge Format

Cartridges have a small cross-sectional area, a slow sample processing rate, and a low tolerance to blockage by particles and adsorbed matrix components. For large sample volumes containing suspended particles, discs are likely to function better. Discs provide shorter sample processing times due to their larger cross-sectional area and decreased pressure drop, enabling higher sample flow rates to be used. The larger cross-sectional area also reduces problems with plugging. For example with a high particle burden, discs with integral or separate depth filters are available, as well as different materials that can be added to the surface of the disc as filter aids.

Because of the low packing density of typical cartridge devices, longer sorbent beds than are needed for extraction are used to compensate for reduced retention resulting from channelling. Increased bed mass results in increased non-specific matrix adsorption and dirtier extracts. The use of smaller particles and the greater mechanical stability of discs reduces channelling, and the optimized use of bed mass results in a cleaner background and lower interferences due to reduced matrix adsorption. For small sample sizes it is easier to miniaturize discs than cartridges, and several disc devices (e.g. microdiscs, pipette tips, etc.) that contain only a few milligrams of sorbent for processing small samples are available. Immoblilized analytes on microdiscs facilitate integrated sample processing techniques such as in-vial extraction and on-disc derivatization.

Inorganic Oxide Adsorbents and their Applications

The most important adsorbents for extraction and matrix simplification are silica gel, alumina, Florisil and diatomaceous earths. Silica gel, prepared from sodium silicate using the sol-gel procedure, is the most widely used general-purpose adsorbent. Silica gels used for solid-phase extraction have surface areas of about 300–800 $m^2 g^{-1}$, pore sizes from 4–10 nm, and an apparent pH of 5.5–7.5. The apparent sorbent pH is characterized as the observed pH of a 5% (w/w) aqueous suspension. Alumina is prepared by the low temperature dehydration ($< 700^{\circ}C$) of alumina trihydrate and is a mixture of γ-alumina with small amounts of α-alumina (less active form) and sodium carbonate. Depending on processing conditions, alumina is available as neutral (pH 7.5 ± 0.5), weakly acidic (pH 6.0 ± 0.5), acidic (pH 4.5 ± 0.5) and basic (pH 9.5 ± 0.5) forms. Adsorbents used for extraction and matrix simplification have a surface area of about 150 $m^2 g^{-1}$ and a pore size of 6 nm. Florisil is a magnesium silicate prepared by precipitation from a mixture of magnesium sulfate and sodium silicate solutions followed by calcining at about 1200°C. It has a surface area of about 250–300 $m^2 g^{-1}$ and an apparent pH of about 8.5. Diatomaceous earths are flux-calcined forms of natural silica with very small surface areas. They are used as a filter aid and as a dispersant for liquid extraction using matrix dispersion techniques (see matrix dispersion).

The general extraction mechanism and applications of the inorganic oxide adsorbents are summarized in **Table 1**. Adsorbent properties that increase retention are a larger surface area and a high activity. Adsorbent activity is controlled by the intentional addition of water to the dried adsorbent prior to use and by drying extracts with anhydrous sodium sulfate, or a similar drying agent, prior to applying the extract to the adsorbent. A small column of

Table 1 General applications of solid-phase extraction

(1) Inorganic oxide adsorbents
- Isolation of low and medium polarity analytes from non-aqueous solutions
- Isolation of cations (alumina and silica) and anions (alumina) from buffered aqueous solutions
- Matrix simplification by fractionation into groups containing a similar number and type of functional group

Examples
- ⇒ Isolation of organochlorine pesticides and polychlorinated biphenyls from transformer oil, animal fats and oils, etc. using Florisil.
- ⇒ Isolation of lipids by chromatography over silica gel using chloroform to elute simple lipids, acetone to elute glycolipids and methanol to elute phospholipids.
- ⇒ Group fractionation of polycyclic aromatic compounds (hydrocarbons, *N*-containing and *OH*-containing) in synthetic fuels over alumina using a step solvent gradient.
- ⇒ Isolation of paraquat and diquat from high moisture crops in a pH 9 aqueous extract using silica gel
- ⇒ Mycotoxins in feeds using silica gel
- ⇒ Pesticides in foods, feeds and soil extracts; alkaloids, pigments and flavour compounds from plants; sugars and caffeine in cola beverages, inorganic anions and organic acids in aqueous solution using alumina; steroids and vitamins from creams and oil-based suspensions.

(2) Low specificity sorbents (aqueous solutions)
- Isolation of neutral and ionizable analytes from aqueous solution. Weak acids and bases by ion suppression. Strong acids and bases using ion pair extraction (alternative to ion exchange)
- Retention increases with solute size and is reduced by polar interactions (particularly hydrogen-bonding) and ionization
- Polar bonded phases provide only weak retention and are not particularly useful unless elution of the analyte is a problem from non-polar sorbents

Examples
- ⇒ Isolation of agricultural and industrial chemicals from surface waters using C_{18}, carbon or poly(styrene-divinylbenzene) (PS-DVB)
- ⇒ Isolation of drugs from biofluids using C_{18}, C_8, PS-DVB or cyanopropyl (CN)
- ⇒ Isolation of macromolecules from biofluids and fermentation broth using C_4
- ⇒ Isolation of pigments and colouring materials from beverages and food extracts using C_{18}
- ⇒ Isolation of carbohydrates and nucleosides from biofluids using AMINO
- ⇒ Isolation of proteins, peptides and surfactants using DIOL

(3) Low specificity sorbents (organic solvents)
- Retention depends on the type and number of functional groups. Solute size is not important

CN	Strong dipole-type interactions and weak hydrogen-bond acidity
AMINO	Strong hydrogen-bond base and weak hydrogen-bond acid. Weak dipole interactions
DIOL	Strong hydrogen-bond acid and weak hydrogen-bond base with significant capacity for dipole-type interactions

Examples
- ⇒ Isolation of polar pesticides from fats and oils
- ⇒ Isolation of polycyclic aromatic compounds from fuel oils
- ⇒ Active ingredients from ointments and suppositories

(4) Ion-exchange sorbents
- In general strong ion exchangers are used to isolate weak acid/bases of opposite charge and weak ion exchangers strong acid/bases
- Retention selectivity can be adjusted by manipulating the sample pH and ionic strength
- Choice of competing ion, its concentration and eluent pH controls selectivity for matrix simplification and elution
- Isolation of macromolecules in an active form may require special non-denaturing sorbents based on cellulose, agarose or dextran

Examples
- ⇒ Isolation of carboxylic, sulfonic and phosphoric acids, phenols, amines and inorganic ions from water
- ⇒ Isolation of amino acids, organic acids, nucleosides and nucleotides from biofluids
- ⇒ Isolation of organic acids and bases from coal-derived and synthetic fuels
- ⇒ Isolation of organic acids, phenols and amines from wine, fruit juices and food extracts

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sodium sulfate connected in tandem with the adsorbent cartridge can be used as an additional precaution. The Brockmann scale (based on the relative retention of test dyes, see **Table 2**) provides a widely used standardized scale of adsorbent activity. Adsorbents of defined activity are prepared by adding a known amount of water to the adsorbent, shaking to avoid clumping, and then allowing the adsorbent to equilibrate overnight in a closed container. Analyte properties that increase retention depend on the number and type of functional groups present. Hydrogen-bonding functional groups are strongly retained, those with a significant dipole-character are retained to a lesser extent, and polarizable functional groups are the least retained. Irreversible adsorption and catalytic degradation of sensitive analytes can occur on all inorganic oxide adsorbents and is a source of low recovery for some analytes. Alumina and silica can function as selective ion exchange sorbents with buffered aqueous samples (see Table 1).

Table 2 Standardization of adsorbent activity

Brockmann activity grade	*Percentage of water (w/w)*		
	Alumina	*Silica gel*	*Florisil*
I[1]	0	0	0
II	3	5	7
III	6	15	15
IV	10	25	25
V	15	38	35

[1]Activate sorbents by heating alumina at 400°C for 8–12 h, silica gel at 180°C for 8–12 h, and Florisil at 130°C for 8–12 h.

Coating silica or alumina with chemical reagents, such as sulfuric acid, sodium hydroxide, alkaline potassium permanganate, silver nitrate, etc., is used to improve the selectivity of the isolation of some analytes from their matrix. Silver nitrate, for example, improves the isolation of olefins from hydrocarbons due to formation of charge-transfer complexes. Acids can be used for the selective isolation of bases and vice versa. Silica impregnated with 2,4-dinitrophenylhydrazine is widely used for the selective isolation of volatile ketones and aldehydes from air for analysis by high pressure liquid chromatography.

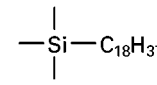
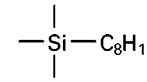
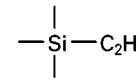
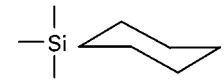
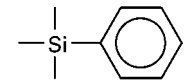
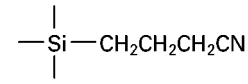
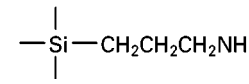
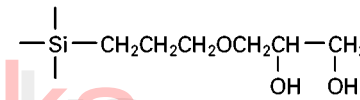
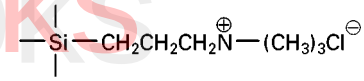
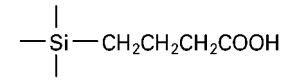
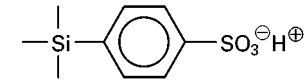
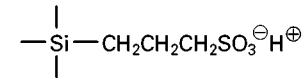
Low Specificity Sorbents and Their Applications

Low specificity sorbents include silica-based, chemically bonded sorbents, macroreticular porous polymers and various forms of carbon. Silica-based, chemically bonded sorbents are derived from materials developed for high pressure liquid chromatography. They are generally prepared by reaction of monofunctional or trifunctional silanes with silica gel followed by end-capping in some cases. Trifunctional reagents result in sorbents with a polymeric-bonded layer of higher carbon loading and greater acid stability and are the more common type of sorbent in general use. Chemically bonded sorbents can be prepared with a wide range of bonding densities, pore sizes and functional group types. Some common examples are given in **Table 3**. Chemically bonded sorbents with large surface areas, long alkyl chains and high phase loading maximize retention of small analytes from aqueous solution, while wide-pore materials with low phase loading and short alkyl chains are generally used to isolate macromolecules. Chemically bonded sorbents with immobilized polar functional groups are used to isolate analytes from organic solutions, based on their selective interactions with analyte polar functional groups (see Table 1).

The macroreticular porous polymers are copolymers of styrene-divinylbenzene or acrylic esters, prepared by suspension polymerization to yield particles consisting of agglomerates of randomly packed microspheres permeated by a network of holes and channels (**Table 4**). They are used exclusively for extraction from aqueous solution and are more retentive than most chemically bonded phases. They possess a high sample capacity and are frequently used in large-scale isolation studies and for the purification of industrial products. Tenax®, a polymer based on 2,6-diphenyl-4-phenylene oxide, revolutionized the sorbent trapping of volatile organic compounds from air and the purge-and-trap analysis of volatile organic compounds in water. It exhibits strong retention of semivolatile organic compounds ($>C_7$) at room temperature with little adsorption of water vapour and can be rapidly heated to high temperatures, without thermal breakdown, for the recovery of analytes by thermal desorption. Since no single adsorbent is ideal for trapping all analytes it is common practice to use cartridges packed with several adsorbent beds in series, so that a broad range of compounds with different molecular weight and polarity can be trapped on a single cartridge. Besides Tenax, different forms of carbon, silica gel and liquid-coated sorbents are used. In a multiple bed cartridge, each bed protects the next, increasingly active bed, by preventing compounds from being held so strongly that they cannot be desorbed quickly without decomposition. During thermal desorption the carrier gas passes through the trap in the reverse direction to the sample flow and the desorbed compounds are swept onto the separation column in a gas chromatograph. A cryogenic interface may be used to refocus the desorbed sample to improve the chromatographic separation. The complete processes of desorption and separation can be automated for sample cartridges stored in an autosampler.

The common forms of carbon used in solid-phase extraction are granular activated carbon, graphitized carbon blacks and carbon molecular sieves. Granular activated carbons are prepared by the low temperature oxidation of vegetable charcoals. They have large surface areas (300–2000 $m^2\,g^{-1}$), a wide pore size distribution, and a heterogeneous surface containing active functional groups. Their use in solid-phase extraction is largely confined to the isolation of dissolved organic compounds in surface waters, and as the sorbent material in personal monitors for sampling workplace atmospheres. The most common form of personal monitor makes use of a sorbent cartridge filled with activated charcoal in conjunction with a small pump to maintain a fixed flow of air through the cartridge. Trapped volatile compounds

Table 3 Structures of silica-based chemically bonded sorbents

Type	*Functional group*	*Structure*
C_{18}	Octadecyl	
C_8	Octyl	
C_2	Ethyl	
CH	Cyclohexyl	
PH	Phenyl	
CN	Cyanopropyl	
NH_2	Aminopropyl	
DIOL	2,3-Dihydroxypropoxypropyl	
SAX	Trimethylaminopropyl (quaternary amine)	
CBA	Carboxypropyl	
SCX	Benzenesulfonic acid	
PRS	Propylsulfonic acid	

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are then eluted with carbon disulfide or another solvent, or can be thermally desorbed by microwave heating, for separation by gas chromatography. Poor reproducibility of activated carbons and their variable chemical and catalytic activity result in limited laboratory use. Graphitized carbon blacks are more refined and generally nonporous, with surface areas between about 5–100 $m^2\,g^{-1}$. They are used primarily for the room temperature trapping of volatile organic compounds ($>C_4$), either separately or in combination with Tenax®. Carbon molecular sieves have small pores and large surface areas ($>500\ m^2\,g^{-1}$ with some $>1200\ m^2\,g^{-1}$). They are used primarily for the room temperature trapping of volatile organic compounds (C_1 and C_2), usually as a component of a multiple-bed sorbent trap for air sampling and purge-and-trap analysis.

Foamed polyurethanes, composed of agglomerated spherical micrometer-sized particles bonded to one another in a rigid and highly permeable structure, are suitable for sampling semivolatile organic compounds (e.g. airborne pesticides and polychlorinated biphenyls) at high flow rates. They are frequently used in conjunction with high-volume air samplers on

Table 4 Characteristic properties of some macroreticular porous polymer sorbents

Amberlite sorbents	*Mean pore diameter (nm)*	*Specific surface area ($m^2\ g^{-1}$)*	*Pore volume ($mL\ g^{-1}$)*	*Sample molecular weight limit*
XAD-2 (STY-DVB)	9	300	0.65	20 000
XAD-4 (STY-DVB)	4	725	0.98	
XAD-7 (MMA)	9	450	1.14	60 000
XAD-16 (STY-DVB)	10	800	1.82	40 000
XAD-2010 (STY-DVB)	28	660	1.80	
DAX-8 (MMA)	22.5	160	0.79	150 000

STY-DVB, styrene-divinylbenzene; MMA, methylmethacrylate.

account of their low pressure drop compared with standard sorbent cartridges. They are used less frequently for water analysis where macroreticular porous polymers are considered a better choice.

Compound and Class-specific Sorbents and their Applications

Various forms of selective sorbents for solid-phase extraction based on ion exchange, bioaffinity, molecular recognition, and restricted access are used to supplement the general class of sorbents discussed above. Ion exchange is used to isolate ionizable compounds (usually) in aqueous solution with sorbents containing fixed ionic sites of opposite charge to the analytes of interest. Ion-exchange sorbents are usually classified as weak or strong depending on the identity of the ionic group and whether its charge is independent of the sample pH (strong ion exchanger) or can be manipulated by changing the pH (weak ion exchanger). Some examples of typical silica-based ion-exchange sorbents are indicated in Table 3. Ion-exchange sorbents with a porous polymer backbone are also commonly used and have a higher exchange capacity and a wider pH-operating range than silica-based sorbents. For many applications either silica-based or porous polymer ion-exchange sorbents with the same immobilized ionic groups can be used interchangeably, although, because of non-specific adsorption of matrix components, the chemical background of the extracts might be different. Ion-exchange sorbents are particularly attractive for the isolation of ionizable substances since the neutral molecules, which may interfere in the final chromatographic analysis, are easily rinsed from the sorbent without affecting the recovery of the ionized components. Mixed-mode sorbents containing ion-exchange sites and alkyl groups co-bonded to silica in either cartridge or disc format are popular in clinical and pharmaceutical laboratories, where they are used for the isolation of ionized drugs and their metabolites from biological fluids. Standard protocols using mixed-mode sorbents have been developed for the isolation of most drugs of abuse (e.g. amphetamines, barbiturates, cocaine, opiates, etc.). The strong retention and the use of efficient rinse solvents results in cleaner extracts compared with single-mode sorbents, suitable for screening by thin-layer chromatography and confirmation by gas chromatography–mass spectrometry.

Resin-bound phenylboronic acids are used for the isolation of compounds with vicinal diol groups such as steroids, catecholamines and nucleotides. Surface-bonded macrocyclic ligands, cryptands, can be used for the selective isolation of metal ions. The cryptands can be synthesized with a variety of cavity sizes suitable for the isolation of different metal ions. The metal ion is sorbed in the cavity of the cryptand until released by elution with a solution of a complexing agent with a high binding constant for the metal.

Immunosorbents have been used for a long time for sample pretreatment in medicine and biology, but more general applications, such as to environmental analysis, are relatively recent. In part, this is due to the difficulty of making antibodies selective to small molecules, as well as a lack of familiarity among analytical chemists of the procedures used to make specific antibodies. Immunosorbents are prepared by covalently bonding a suitable antibody to an appropriate sorbent. A high degree of molecular selectivity is obtained based on the specificity of the antibody–antigen (analyte) interaction. Because specificity is high, immunosorbents are able to isolate target analytes from complex matrices in a single step with minimal co-extraction of matrix interferences. By taking advantage of cross-reactivity, class-specific immunosorbents for the isolation of mycotoxins, phenylurea herbicides and polycyclic aromatic hydrocarbons have been developed. Manufactured immunosorbents have been available for only a short time and the range of products is still narrow. A laboratory familiar with the techniques for raising and isolating antibodies is required.

Molecular imprinting is a technique used for preparing polymers with synthetic recognition sites

having a predetermined selectivity for a specified analyte. The imprint is obtained by arranging polymerizable functional monomers around a template (the analyte). Template–monomer complexes are formed in solution through molecular interactions and subsequently fixed in place by cross-linking. Removal of the template from the resulting polymer matrix creates vacant recognition sites that exhibit affinity for the analyte. For the time being, it is impossible to predetermine the experimental conditions for successful imprinting of target analytes. The template molecule may be difficult to leach from the imprinted polymer, reducing the binding capacity of the polymer, but more seriously, it may lead to contamination of sample extracts. Only a few practical applications using molecularly imprinted polymers for solid-phase extraction have been demonstrated so far, most of which are for the isolation of drugs from biological fluids, but the future for this technology looks very promising. Molecularly imprinted polymers should be easier and cheaper to produce in chemical laboratories than antibodies while, at least in theory, they should be capable of similar specificity.

Restricted access sorbents have been developed for the isolation of low molecular weight compounds, generally drugs, directly from biological fluids with minimum sample pretreatment. They work by preventing access of macromolecules (proteins) to those regions of the sorbent where retention of the analyte occurs. Restricted access to the retentive part of the sorbent is provided by either a physical diffusion barrier, such as a pore diameter, or by a chemical diffusion barrier, such as a polymer network at the outer surface of the particle. In addition, the outer surface of the particles must be non-adsorptive and protein-compatible. Restricted access sorbents are commonly used for automated on-line sample processing in liquid chromatography. In this case, a short precolumn packed with the restricted access material is interfaced to a separation column by a six-port switching valve. The biofluid is injected directly onto the precolumn, which retains the analytes of interest. Potentially interfering sample constituents are then flushed to waste. Macromolecules (proteins) pass through the precolumn unretained and do not interfere in the subsequent separation of the analytes. The analytes retained on the precolumn are eluted on-line to the separation column and detected. Simultaneously the precolumn is reconditioned (or exchanged) before processing the next sample. An important consideration for automated sample processing is the ability of the restricted access sorbent to repeatedly extract the analyte without change in properties or accumulation of sample matrix components.

Sample Processing Considerations

Solid-phase extraction cartridges are available in a range of sizes containing from about 35 mg to 10 g of sorbent, with the 100 mg and 500 mg sorbent cartridges (or discs) being the most widely used for extraction and the larger cartridge sizes for sample clean up. As a rough guide, the sorbed sample capacity of a solid-phase extraction device is about 1–5% of the sorbent mass. The sample volume that can be processed depends primarily on the breakthrough volume of the analyte, the concentration of the analyte matrix, sample flow rate, and the sorbent mass. The sample volume is often selected to conform to the needs of the instrumental detection step, and as instrumental methods of determination have improved in sensitivity, sample volumes have decreased in size. Regulatory authorities often indicate action levels in concentration units, which can also be used to define an adequate sample volume for analysis.

Sorbent selection is based on the considerations summarized in **Figure 6**. The sample solvent (aqueous or organic), the analyte type (non-polar, polar or ionized), and whether it is ionized (strong or weak, acid or base) provides a logical guide for method selection. Organic compounds soluble in polar organic solvents but difficult to dissolve in solvents of intermediate polarity, can be extracted in the reversed-phase mode if they can be reconstituted in aqueous solution.

Sample processing involves four distinct steps. Initially, the sorbent is conditioned with solvent to improve the reproducibility of analyte retention and to reduce the carrythrough of sorbent impurities at the elution stage. The conditioning solvent is then replaced with the same solvent as the sample solvent and the sample passed through the sampling device at a controlled flow rate. Optionally, after the sample has been processed, the sorbent is rinsed with a weak solvent to displace undesired matrix components from the sorbent without displacing the analytes. Finally, the analytes of interest are eluted from the sorbent in a small volume of strong solvent for subsequent determination. Hidden in the above description of events are a number of sub-steps that can dramatically influence analyte recovery if not adequately optimized (**Table 5**). The conditioning step is critically important for processing aqueous samples using particle-loaded membranes. The high surface tension of water combined with the microporosity of the discs results in slow and uneven flow through the discs and low analyte recovery if the discs are not first conditioned with an organic solvent. For large sample volumes, a small amount of the same organic solvent is usually added to the sample to maintain a constant

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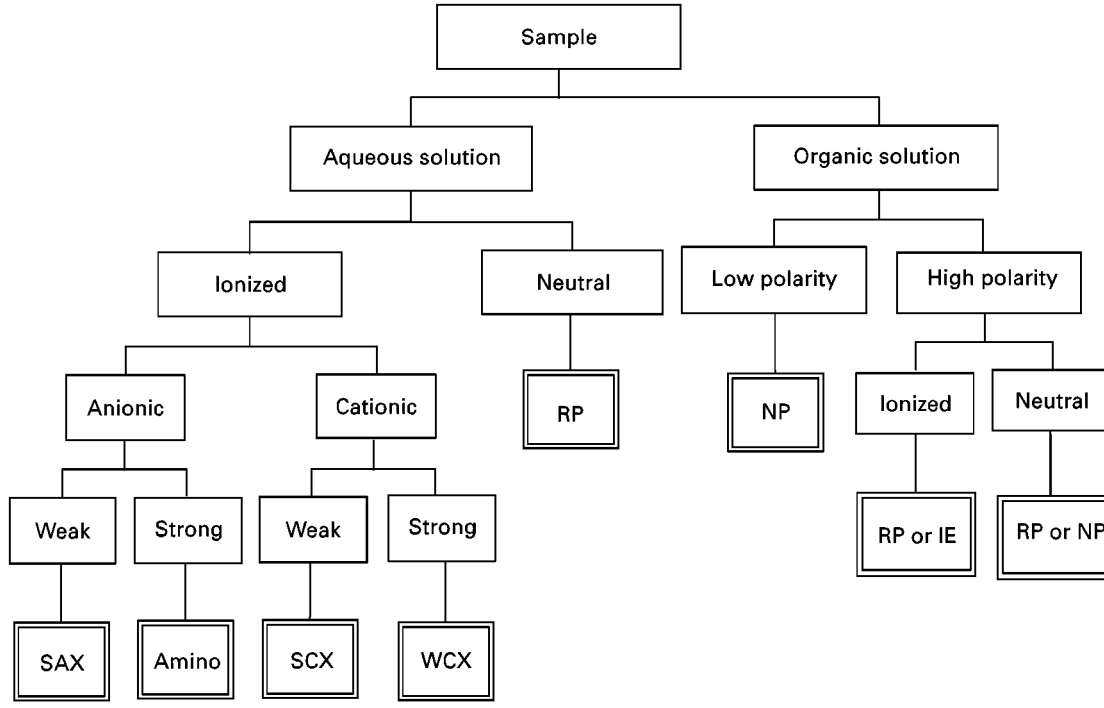

Figure 6 Method development guide for the isolation of organic compounds from liquid samples. SAX, strong anion exchanger; SCX, strong cation exchanger; WCX, weak cation exchanger; RP, reversed-phase sampling mode; NP, normal-phase sampling mode; IE, ion exchange sampling mode.

sample flow rate. The drying step between processing aqueous samples and eluting the retained analytes with a water-miscible organic solvent is also important. The purpose of the drying step is to reduce the volume of water co-eluting from the sampling device permitting further concentration of the eluent by the gas-blow down method. Drying, by suction or storage in a vacuum desiccator, should be sufficient to remove water trapped in the pores, but excessive drying can result in low analyte recovery from evaporation or inefficient elution. A new porous polymer sorbent, prepared by copolymerization of divinylbenzene and *N*-vinylpyrrolidone (Oasis HLB), and solvated by water alone, has been suggested as a solution to this problem. Recently, it was shown that all sample-processing steps are amenable to computer-aided method development, replacing the traditional experimental trial-and-error approach by fast computer simulations.

Automation

Automation provides a better utilization of laboratory resources, unattended and out-of-hours operation and improved precision compared with manual methods. Common approaches to automation differ significantly. Using robotics, samples are processed (usually) in a similar manner to manual methods. Using flow-processing schemes, samples are extracted in parallel with computer or microprocessor control of solvent management. Sorbent conditioning, sample condition, solvent selection, rinse and elution steps are performed automatically and can be varied for method development. Positive displacement instead of suction is used for solvent control, and advanced units can be programmed to replace sorbent cartridges to increase sample throughput and inject extracts into different chromatographic instruments. On-line analysers with a direct coupling to chromatographic instruments are widely used. Solid-phase extraction using short precolumns and a switching valve interface is a routine method for analysis by liquid chromatography. Advanced systems even allow programmed replacement of the sorbent cartridges and unattended 24-hour operation. The recovery and separation steps of purge-and-trap and sorbent trapping of volatile organic compounds from air are easily automated using thermal desorption with cold trapping, if required, for the direct injection of analytes into a gas chromatograph. Major strides have been made in the on-line solid-phase extraction of water samples with solvent desorption directly into a gas chromatograph. This method is not far from becoming routine today.

Table 5 Experimental variables that influence recovery of analytes by solid-phase extraction

- Conditioning solvent (typically 3–5 bed volumes)
  - ⇒ Ensures reproducible retention and flow. Critical step for particle-loaded membranes
  - ⇒ Helps to minimize contamination of extracts by sorbent impurities
  - ⇒ Replace by sample solvent before processing sample
- Flow rates (typical range 0.2–1.5 mm s^{-1})
  - ⇒ More critical for cartridges than discs due to their variable and heterogeneous packing density (channelling)
  - ⇒ More critical when the sample volume exceeds the breakthrough volume as typical sampling devices provide too few theoretical plates for flow-independent retention
- Sample properties
  - ⇒ Dilute viscous samples with a weak low viscosity solvent to reduce sample processing time
  - ⇒ Remove excessive particle matter by filtration or centrifugation to maintain a constant sample-processing rate. Concentrated hydrochloric acid is effective for dissolving inorganic particles in water samples
  - ⇒ Add small volume of organic solvent (1–3% v/v) to large volume water samples to ensure sorbent remains solvated and to maintain a constant (fast) sample-processing rate. Important for particle-loaded membranes
  - ⇒ Adjust pH to reduce ionization of weak acids and bases for reversed-phase sampling
  - ⇒ Maintain ionic strength approximately constant for samples and standards with reversed-phase sampling conditions. Ionic strength is a critical parameter for ion-exchange extraction
  - ⇒ Deproteination of biofluids may be required for acceptable recovery of low molecular weight analytes for reversed-phase sampling
  - ⇒ Precipitation of inorganic acids (sulfate, phosphate, etc.) by barium hydroxide is sometimes required for acceptable recovery of organic acids from biofluids using ion-exchange extraction
- Drying time (typically 1–5 min, but sometimes considerably longer)
  - ⇒ Sufficient to remove all sample solvent trapped in the sorbent pores
  - ⇒ Excessive drying may result in low recovery of analytes from evaporation or retention in poorly solvated regions of the sorbent
- Rinse solvent (optional)
  - ⇒ Small volume of intermediate strength solvent to elute matrix components. Analytes remain immobilized on the sorbent
  - ⇒ Biological fluids, plant extracts and soil extracts often require a rinse step but surface waters may not
- Eluting solvent (ideally 2–3 bed volumes but often larger)
  - ⇒ Should be a strong solvent able to displace all analyte from the sorbent in a small volume
  - ⇒ Should normally be volatile and miscible with the sample solvent

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Future developments

Solid-phase extraction is approaching maturity and is a familiar laboratory operation for many analytical chemists. Advances are expected in the area of specific sorbents based on molecular imprinting or bioaffinity designed for the convenient isolation of target compounds in complex matrices. Advances are also expected in the use of computer-aided method development for the prediction of sampling and recovery conditions by simulation to replace tedious experimental trial-and-error approaches. A wider use and further development of automated solid-phase extraction systems can be expected, particularly in those industries where high sample throughput or round-the-clock process monitoring are important.

See also: **II/Affinity Separation:** Immobilised Boronates and Lectins; Imprint Polymers. **Extraction:** Solvent Based Separation. **III/Airborne Samples:** Solid Phase Extraction. **Immunoaffinity Extraction. Immobilised Boronic Acids:** Extraction. **Molecular Imprints for Solid-Phase Extraction. Restricted-Access Media: Solid-Phase Extraction. Solid-Phase Extraction with Cartridges. Solid-Phase Extraction with Disks. Solid-Phase Matrix Dispersion: Extraction. Sorbent Selection for Solid-Phase Extraction. Appendix 2 /** Essential Guides to Method Development in Affinity Chromatography.

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Solid-Phase Microextraction

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Introduction

Solid-phase microextraction (SPME) was introduced as a solvent-free sample preparation technique in 1990. The basic principle of this approach is to use a small amount of the extracting phase (usually less than 1 μL) compared to the sample matrix. Sample volume can be very large, when the investigated system, for example air or lake water, is sampled directly. The extracting phase can be either a high molecular weight polymeric liquid, similar in nature to chromatographic stationary phases, or it can be a solid sorbent, typically of a high porosity to increase the surface area available for adsorption.

To date the most practical geometric configuration of SPME utilizes a small fused silica fibre, usually coated with a thin film of polymeric phase. The fibre is mounted for protection in a syringe-like device (**Figure 1**A). The analytes are absorbed or adsorbed by the fibre coating (depending on the nature of the coating) until an equilibrium is reached in the system. The amount of an analyte extracted by the coating at equilibrium is determined by the magnitude of the partition coefficient (distribution ratio) of the analyte between the sample matrix and the coating material.

In SPME, analytes typically are not exhaustively extracted from the matrix. However, equilibrium methods are more selective because they take full advantage of the differences in extracting phase/matrix distribution constants to separate target analytes from interferences. Exhaustive extraction can be achieved in SPME when the distribution constants are large enough. This can be accomplished for most compounds by cooling the fibre coating. This concept was tested using a piece of microtubing coated on the outside instead of a solid rod and supplying liquid carbon dioxide into the tube to achieve an internally cooled fibre. In exhaustive extraction, selectivity is sacrificed to obtain quantitative transfer to target analytes into the extracting phase. One advantage of this approach is that, in principle, it does not require calibration, since all the analytes of interest are transferred to the extracting phase. On the other hand, the equilibrium approach usually requires calibration through the use of surrogates or standard addition to quantify the analytes and compensate for matrix-to-matrix variations and their effect on distribution constants.

Since equilibrium rather than exhaustive extraction occurs in microextraction methods, SPME is ideal for field monitoring. It is unnecessary to measure the volume of the extracted sample and therefore the SPME device can be exposed directly to the investigated system for quantitation of target analytes. Thin coatings of extracting phase result in fast separations. In addition, extracted analytes are introduced to the analytical instrument inlet system by simply placing the fibre in the desorption unit (Figure 1B and 1C). This convenient, solvent-free sample introduction process facilitates sharp injection bands and rapid separations. These features of SPME result in the integration of the first steps in the analytical process: sampling, sample preparation and introduction of extracted mixture to the analytical instrument. For example, total analysis time in field applications can be as low as a few minutes when portable instrumentation is used.

The equilibrium nature of the technique also facilitates speciation in natural systems since the presence of a minute fibre, which removes small amounts of target analytes, is not likely to disturb the system. Because of the small size, coated fibres can